28. (New) The affinity fluorescent protein of Claim 27 wherein the mutated GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro).

sub C4 B5 cont'd

29. (New) An isolated affinity fluorescent protein expression cassette comprising a modified green fluorescent protein (GFP) nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein the modified GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro) and a hexapeptide LEPRAS (SEQ ID NO: 1).

REMARKS

Claim amendments

Claims 11-15 are pending. Claims 11 and 14 have been amended to add the term "isolated". Support for the term "isolated" can be found, for example, on page 25, line 23 of the specification. Claim 12 has been amended to ass "(SEQ ID NO: 1)". Support for the amendment can be found, for example, on page 3, line 16 of the specification. Claims 11, 13, 14 and 15 have been amended to recite the full term for the acronyms "GFP", "Gln", "Lys", "Glu", "Asp", "Ser" and "Pro". New Claims 27-29 have been added.

Request For Corrected Filing Receipt

Applicants direct the Examiner's attention to the Request For Corrected Filing Receipt (the Request) being filed concurrently. The Request is being filed to correct the "Continuing Data as Claimed by Applicant" section of the filing receipt.

Priority

The Examiner notes that "[p]rovisional application no. 60/061,801 was filed more than one year to the filing of the instant application" and states that "[b]enefit to this application is not granted" (Office Action, page 2).

Applicants agree with the Examiner. Applicants do not claim the benefit of the filing date of U.S. Application No. 60/061,801. The "RELATED APPLICATION(S)" section of the referenced application indicates that the referenced "application *is related* to Application No. 60/061,801" and "*claims the benefit* of U.S. Application No. 60/146,438" (specification, page 1, lines 3-5, emphasis added). Applicants are filing concurrently a Request For Corrected Filing Receipt to correct the "Continuing Data as Claimed by Applicant" section of the filing receipt.

Information Disclosure Statement

The Examiner states that the "information disclosure statement filed 3/22/01-Paper #6, has been considered as to the merits to first action" (Office Action, page 3). Accordingly, the Examiner initialed and returned Applicants PTO 1449 form.

However, Applicant also submitted U.S. Provisional Application No. 60/061,801 which was listed on page 2 of the transmittal letter filed with PTO form 1449. Applicants respectfully request that the Examiner initial and return page 2 of the transmittal letter filed with PTO form 1449 to indicate that U.S. Provisional Application No. 60/061,801 has been considered.

Oath/Declaration

The Examiner states that a "new oath or declaration is required because provisional application number 60/061,801, filed 10/14/1997 is not listed under 35 USC 119(e)" (Office Action, page 3).

Applicants respectfully disagree. As pointed out above, Applicants do not claim priority to U.S. Provisional Application No. 60/061,801, filed October 14, 1997 because as noted by the Examiner, the application "was filed more than one year to the filing of the instant application" (Office Action, page 2). Therefore, a new oath or declaration is not required.

Specification

The Examiner notes the use of trademarks in the application and indicates that they should be capitalized.

The specification has been amended to capitalize trademarks.

Objection to Claims 11-15 under 37 C.F.R. §1.821(d)

Claims 11-15 are objected under 37 C.F.R. §1.821(d) "for failing to recite the SEQ ID NOS in the claims" (Office Action, page 4).

Claim 14 has been amended to recite "SEQ ID NO: 1". Claims 11 and 14 refer to green fluorescent protein (GFP). In the specification as filed, Applicants teach that the "original plasmid pEGFP purchased from Clontech (accession #U76561)" was used to prepare the claimed affinity fluorescent protein expression cassette comprising a modified GFP nucleic acid sequence (specification, page 16, lines 14-15). Accession #U76561 which provides the pEGFP complete sequence is well known and readily available to those of skill in the art, thus, a sequence identification number is not needed.

Rejection of Claims 11-15 under 35 U.S.C. §101

Claims 11-15 are rejected under 35 U.S.C. §101 as being directed to non-statutory subject matter. The Examiner states that the invention "reads on an affinity fluorescent expression cassette/vector/molecule, which includes naturally occurring as well as synthetic compositions" (Office Action, page 4). The Examiner suggests incorporating the term "isolated" or "purified" in the claims.

The term "isolated" has been added to Claims 11 and 14, thereby obviating the rejection. Support for the term "isolated" can be found, for example, on page 25, line 23 of the specification.

Rejection of Claims 11-15 under 35 U.S.C. §112, second paragraph

Claims 11-15 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 5). The Examiner states that "Claims 11-15 are vague and indefinite in utilizing the following acronyms: GFP, LEPRAS, Gln, Asp, Ser, Pro, etc" (Office Action, page 5).

Claims 11-15 have been amended to recite the full term for the acronyms "GFP", "Gln", "Lys", "Glu", "Asp", "Ser" and "Pro". However, "LEPRAS" is not an acronym; it represents an amino acid sequence. In order to clarify this, "SEQ ID NO: 1" has been added to the claims.

Rejection of Claims 11-15 under 35 U.S.C. §103(a)

Claims 11-15 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Tsien et al. (WO 97/28261) in view of Tsien et al. (USC Patent #6,066,476) and further in view of (WO 98/36081)" (Office Action, page 6). The Examiner states that Tsien *et al.* (WO 97/28261) "teach tandem fluorescent protein constructs including GFPs" (Office Action, page 6). The Examiner further states that Tsien *et al.* employ a "first polypeptide binding domain-donor fluorescent protein construct, an acceptor fluorescent protein moiety, and a second polypeptide binding domain-a linker moiety coupling the donor and acceptor (tandem fluorescent protein constructs)"; that "[o]ne of the components of this tandem fluorescent protein construct comprises a cleavage recognition site for an enzyme"; that "[f]luorescence resonance energy transfer measurements can be determined when the donor moiety is excited"; and that "[e]nzymatic activity assays are utilized to detect both *in vitro* and *in vivo* evaluations" (Office Action, page 6). The Examiner states that Tsien *et al.* (6,066,476) teach that "modification in the sequence of Aequorea wild-type GFP could provide products with different excitation and emission spectra's" and that "[s]everal different restriction endonucleases sites are discussed" (Office Action, page 6). The Examiner notes that Tsien *et al.* (WO 97/28261) and Tsien *et al.* (6,066,476) "differ from the instant invention in not specifically teaching the reaction sites recited in the claims" (Office Action, page 6). The Examiner cites Miesenbock *et al.* as disclosing "affinity flourescent protein expression molecules comprising modified GFP" and provides examples of amino acid substitutions. It is the Examiner's opinion that:

> [i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize various modified GFP sequence as taught by Miesenbock et al. in the GFP tandem constructs of Tsien et al. (WO 97/28261) in view of the modified GFPs of Tsien et al. to produce affinity fluorescent protein molecules because such restriction site modification procedures as taught by Miesenbock et al. are well known in the art. A person of ordinary skill in the art would have a reasonable expectation of success utilizing endonuclease restriction in GFP sequence production as taught by Miesenbock et al. because fluorescent molecules were taught to be attractive as receptor molecules in many assay systems because of their high sensitivity and ease of quantification (Office Action, page 7).

The Examiner further states that:

> [a] person of ordinary skill in the art would have been motivated to employ modified GFPs because Miesenbock et al. taught that such restriction modifications would further enhance the ability of the molecules to detect changes in the microenvironment. . . . With respect to the particular restrict sites, unless the result obtained in the instant application is a significant and unexpected difference over the prior art, it would have been prima facie obvious for one of ordinary skill in the art to modify GFP at known restriction sites in order to produce the most optimal configuration useful in the given parameters (Office Action, page 7).

Applicants respectfully disagree. Applicants' claimed invention relates to an isolated affinity fluorescent protein expression cassette comprising a modified GFP nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein *the modified GFP sequence comprises a recombinant peptide which comprises* ***restriction endonuclease sites introduced at a location of the GFP molecule*** selected from the group consisting of: between Gln157 and Lys158, between Glu172 and Asp173 and both of the aforementioned locations.

35 U.S.C. § 103 requires both (1) that "the prior art would have suggested to the person of ordinary skill in the art that they should . . . carry out the claimed process"; and (2) that the prior art should establish a reasonable expectation of success (*In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991)). "Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *Id.* The combined teachings of Tsien *et al.* (WO 97/28261) in view of Tsien *et al.* (U.S. Patent 6,066,476) and further in view of Miesenbock *et al.* (WO 98/36081) do not teach or suggest introducing restriction endonuclease sites at a location of a GFP molecule selected from the group consisting of: between Gln157 and Lys158, between Glu172 and Asp173 and both of the aforementioned locations.

Applicants teach that fluorescent proteins have "fluorescent insensitive sites into which can be introduced a ligand-activated protein binding site, thereby producing a modified fluorescent protein, and wherein the modified fluorescent proteins displays an altered spectral property when the binding site is engaged with ligand" (specification, page 8, lines 26-30). Applicants produced affinity fluorescent proteins (aFPs) by "introducing heterologous amino acid sequences (e.g., peptides or epitopes) encoding particular ligand binding sites, into select

regions of the wild type molecule which correspond to loops present on the surface of a fluorescent protein" (specification, page 8, lines 16-19). Applicants teach that:

> [t]o facilitate the identification of fluorescence insensitive sites which can accommodate the presence of a binding site and/or the production aFPs, an affinity fluorescent protein cassette can be created by introducing a small synthetic test peptide comprising one or more appropriate restriction enzymes sites at candidate locations in the sequence of a fluorescent protein such as GFP. After identifying regions (e.g., guest loops) of the fluorescent protein which can tolerate the introduction of the test peptide without a lose [sic] of fluorescent intensity, the restriction sites can be used for the introduction of heterologous amino acid sequences, or non-protein moieties which embody the desired binding site (specification, page 9, lines 11-19).

In contrast, Tsien *et al.* (WO 97/28261) teach "[t]andem fluorescent protein constructs in which ***two fluorescent protein moieties*** capable of exhibiting FRET [fluorescence resonance energy transfer] are coupled through a linker to form a tandem construct" (Tsien *et al.* WO/9728261; page10, line 32 - page 11, line 1, emphasis added). Tsien *et al.* (WO 97/28261) further teach that the "linker moiety is, preferably, a peptide moiety, but can be another organic molecular moiety" and that "[r]upture of the linker moiety results in separation of the fluorescent protein moieties that is measurable as a change in FRET" (Tsien *et al.* (WO 97/28261), page 23, lines 3-12). In a particular embodiment, the linker is a "cleavage site for a protease of interest" (Tsien *et al.* (WO 97/28261), page 12, lines 18-19). Tsien *et al.* (WO 97/28261) do not teach introducing a recombinant peptide which comprises restriction endonuclease sites introduced at a location in a single GFP molecule.

Tsien *et al.* (U.S. Patent 6,066,476) teach that "particular modifications in the polypeptide sequence of an Aequorea wild type GFP . . . lead to formation of products having markedly different excitation and emission spectra from corresponding products derived from wild-type GFP" (Tsien *et al.* (U.S. Patent 6,066,476), column 2, lines 24-28). Tsien *et al.* (U.S. Patent 6,066,476) subjected Aequorea GFP cDNA to "random mutagenesis by hydroxylamine or polymerase chain reaction" (Tsien *et al.* (U.S. Patent 6,066,476), col. 3, lines 52-54). The modified GFP in Tsien *et al.* (U.S. Patent 6,066,476) have one or more point mutations and exhibit "significant alterations in the ratio of the two main excitation peaks" (Tsien *et al.* (U.S. Patent 6,066,476), column 3, lines 62-63), exhibit fluorescence "at different wavelengths" (Tsien

et al., (U.S. Patent 6,066,476), column 4, lines 53-54), or exhibit "substantially more intense fluorescence per molecule than the wild type molecule" (Tsien *et al.*, (U.S. Patent 6,066,476), column 5, lines 12-14). Tsien *et al.* (U.S. Patent 6,066,476) state the modified GFPs can be for labelling polypeptides of interest, but do not mention "different restriction endonuclease sites" as the Examiner indicates (Office Action, page 6). Tsien *et al.* (U.S. Patent 6,066,476) do not teach introducing a recombinant peptide which comprises restriction endonuclease sites introduced at a location in a GFP molecule.

Miesenbock *et al.* teach "hybrid molecules comprising a targeting region and a reporter region capable of participating in a reaction resulting in an optically detectable signal when the hybrid molecule encounters a change in the microenvironment" and that a "linker comprising at least one amino acid may also be interposed between the targeting and reporter regions" (Miesenbock *et al.*, page 18, lines 2-5 and 20-21). The reporter region of the hybrid molecules "may be any molecular moiety that participates in a bioluminescent, chemiluminscent, fluorescent, or fluorogenic reaction", such as GFP, and preferably, the reporter "is an amino acid sequence which is co-expressed as a fusion protein with the targeting amino acid sequence" (Miesenbock *et al.*, page 23, lines 30-32; page 25, lines 11-13). In addition, Miesenbock *et al.* describe environment-sensitive GFP mutants, termed pHluorins, which were generated using various amino acid substitutions shown in Table 2 (Miesenbock *et al.*, page 48). However, Miesenbock *et al.* do not teach introducing a recombinant peptide which comprises restriction endonuclease sites introduced at a location in a GFP molecule.

The combined teachings of the cited references do not teach or suggest introducing a recombinant peptide which comprises restriction endonuclease sites ***within*** a GFP molecule for use as an affinity fluorescent protein expression cassette to one of ordinary skill in the art at the time the invention was made. The cited references discuss modified GFPs which include one or more amino acid substitutions, but do not further teach introducing a recombinant peptide which comprises restriction endonuclease sites ***within*** a GFP molecule. The Examiner states that "[s]everal different restriction endonuclease sites are discussed" in Tsien *et al.* (U.S. Patent 6,066,476), however, Applicants fail to find such a teaching.

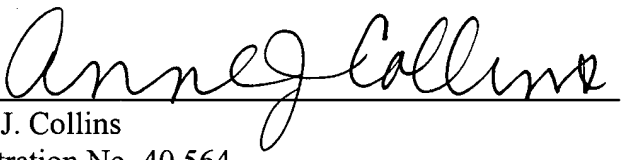
Clearly, Tsien *et al.* (WO 97/28261) in view of Tsien *et al.* (U.S. Patent No. 6,066,476) and further in view of Miesenbock *et al.* (WO 98/36081) do not render obvious Applicants' claimed invention.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By 

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Dated: May 6, 2002

MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 9, lines 11 through page 10, line 6 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

To facilitate the identification of fluorescence insensitive sites which can accommodate the presence of a binding site and/or the production aFPs, an affinity fluorescent protein cassette can be created by introducing a small synthetic test peptide comprising one or more appropriate restriction enzymes sites at candidate locations in the sequence of a fluorescent protein such as GFP. After identifying regions (e.g., guest loops) of the fluorescent protein which can tolerate the introduction of the test peptide without a [lose] loss of flourescent intensity, the restriction sites can be used for the introduction of heterologous amino acid sequences, or non-protein moieties which embody the desired binding site. For example, the hexapeptide LEPRAS (SEQ ID NO: 1) which contains three restriction enzyme sites (XhoI-AvrII-NheI) was useful for identifying flourescent insensitive sites in the GFP molecule. Alternatively, other test peptides can be designed which exhibit characteristics such as hydrophobicity and charge in common with either the native loop or with the heterologous amino acid sequence or moiety selected for introduction into the fluorescent protein. Affinity and specificity of binding of an aFP of the present invention can be further tailored by additional modification of the same loop, for example, by introducing two or more binding sites (e.g., linear or cyclic peptides) in tandem at a single location, or by introducing the same binding site at distinct locations. For example, two binding sites can be introduced at the position between Gln157 and Lys158 (e.g., 157HA2 or 157HA2) or at the position between Glu172 and Asp173 (e.g.,172HA,172HA2). Alternatively, a single copy of each binding site can be introduced at two or more distinct sites (e.g., 157HA/172HA). Depending on the nature of the target ligand, affinity of the binding of an aFP of the present invention may also be enhanced by introducing an additional binding sites at either, or both, the N-terminus and C-terminus (e.g., 157/CHA) of the GFP molecule.

Replace the paragraph at page 16, lines 14 through 19 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Expression Vectors. The original plasmid pEGFP purchased from Clontech (accession #U76561). Vector pProEX Hta from Life Technologies (cat.#10711-018) containing (His)6 tag at the amino-terminus for affinity purification. Restriction enzymes and DNA ligases were purchased from New England Biolabs (Beverly, MA). PCRs were performed on [RoboCycler Gradient] ROBOCYCLER GRADIENT 96 (Stratagene) using PCR Supermix (Life Technologies). DNA purification and gel extraction were done using QLAGEN kits.

Replace the paragraph at page 18, lines 9 through 14 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Absorption, Excitation, and Emission Spectra. The absorption spectra were collected on an AVIV Model 118DS spectrophotometer. (AVIV Associates, Inc., Lakewood, NJ) at 25°C. Excitation and emission spectra were recorded on a [Fluorolog] FLUOROLOG 3-22 spectrofluorimeter (Instruments S.A., Inc., Edison, NJ) at 25°C. The instrument parameters are the following: slit of 2.5 nm, integration time of 0.5 second, interval of 1 nm, and PMT 950V.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

11. (Amended) An isolated affinity fluorescent protein expression cassette comprising a modified [GFP] green fluorescent protein (GFP) nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein the modified GFP sequence comprises a recombinant peptide which comprises restriction endonuclease sites introduced at a location of the GFP molecule selected from the group consisting of: between [Gln] glutamine (Gln) 157 and [Lys] lysine (Lys)158, between [Glu] glutamic acid (Glu)172 and [Asp] aspartic acid (Asp)173 and both of the aforementioned locations.

12. (Amended) The affinity fluorescent protein expression cassette of Claim 11, wherein the recombinant peptide comprises the hexapeptide LEPRAS (SEQ ID NO: 1).

13. (Amended) The affinity fluorescent protein of Claim 11 wherein the mutated [GFP] green fluorescent protein (GFP) comprises a substitution of [Ser147Pro] serine at position 147 of GFP to proline (Ser147Pro).

14. (Amended) An isolated affinity fluorescent protein expression vector comprising a modified [GFP] green fluorescent protein (GFP) nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein the modified GFP sequence comprises a heterologous amino acid sequence introduced at a position of the GFP molecule selected from the group consisting of: between [Gln] glutamine (Gln) 157 and [Lys] lysine (Lys)158, between [Glu] glutamic acid (Glu)172 and [Asp] aspartic acid (Asp)173 and both of the aforementioned locations.

15. (Amended) The affinity fluorescent protein expression vector of Claim 14 wherein the mutated [GFP] green fluorescent protein (GFP) comprises a substitution of [Ser147Pro] serine at position 147 of GFP to proline (Ser147Pro).